IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of

BALINT ET AL

Serial Number: 09/526,106

Filed: April 1, 1998

For: CIRCULARLY PERMUTATED INTERACTION ACTIVATED PROTEINS

Group Art Unit: 1627
Examiner: Thomas Friend

#14
Dupp
8-8-02

PROTEST UNDER 37 C.F.R. § 1.291

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

Pursuant to 37 C.F.R. § 1.291, the undersigned hereby files a protest against pending application 09/526,106 and any other related continuing applications and respectfully requests that the patent office consider the enclosed prior art and arguments presented herein. Protester is aware of the following related numbers: US provisional Serial Numbers 60/135,926 and 60/175,968; and WO 00/71702 and WO 01/51629 which appear to be the corresponding applications related to 09/526,106. The discussion centers around the published claims of the WO's however protester believes that no claims should be allowed regardless of amendments. The undersigned believes that all pending claims of US serial No. 09/526,106 are not patentable.

SUMMARY

In the above-mentioned patent applications, Balint and Her describe the creation of interaction-activated proteins. A review of prior art shows that the strategy, specifications and examples are identical to an existing, broadly enabling invention known as protein-fragment complementation assays (PCA), which is described and claimed in US 6,270,964 (the '964 patent) by Michnick et al. The principles, methods and applications of PCA have now been published in 10 scientific articles (see references) and has been applied to a variety of reporters, cell types, cellular compartments and biological applications. A review of WO 00/71702 shows that the strategy, examples and claims are identical to PCA and are specifically taught by the '964 patent and by the Michnick publications. A review of WO 01/51629 shows that 10 out of the 11 examples are identical to PCA; in fact, only one example (example 11) relates to a circularly permutated interaction-dependent protein. In other words, Balint and Her have simply applied PCA using the concepts, strategies and methods exactly as taught and claimed in the Michnick patent; using a reporter which fits the criteria taught by Michnick as being suitable for PCA; and using the rational design and genetic engineering methods which were described, incorporated by reference, and claimed by Michnick et al.

What is PCA?

PCA represents a complete strategy for identifying and characterizing biomolecular interactions and mapping biochemical pathways. The strategy involves utilizing molecular reporters that allow the direct detection, visualization and quantitation of biomolecular events, such as protein-protein interactions and drug-protein interactions. The strategy can be used to determine the functions of genes in the biological context of a living cell, and has been proven to

work in a variety of cell types including bacterial, yeast, insect, mammalian and plant cells. Further, recent work by Michnick and his colleagues demonstrates that the PCA strategy can be used to screen libraries, such as cDNA or antibody libraries, to map out entire biochemical pathways implicated in human disease, to identify novel mechanisms by which pathways talk to each other, and to assess the actions of drugs on pathways. In addition Michnick has designed and reduced to practice a wide variety of marker proteins in PCA, showing that the PCA principle can be applied to any suitable marker protein.

Over the course of his research, Dr. Michnick utilized the design principles of fragmenting a reporter molecule, such as an enzyme or a protein, resulting in reversible loss of reporter function. He then fused or attached the fragments of the reporter molecule separately to other molecules, such that folding of the reporter from fragments would be driven by an interaction of the molecules that were fused to the fragments. Simultaneous folding and reassociation of the fragments results in reconstitution of the activity of the reporter, generating a signal. The use of polypeptide fragments that are generated by design results in a reporter system that is free of background, in contrast to systems that employ subunits of enzymes - or otherwise naturally complementing polypeptides - which exhibit a degree of complementation even in the absence of fused interacting molecules. The PCA principle is generalizable; it can be applied to any suitable reporter, as taught in the '964 patent, and as demonstrated in the references. This enables the creation of reporters suitable for specific applications, including survival-selection assays in bacterial and mammalian systems; and colorimetric, fluorescent, or luminescent readouts both in living cells and in vitro. The '964 patent also teaches that any method of engineering can be used to create the reporter fragments themselves including rational

design and genetic engineering, and that any protein which generates a detectable signal can be used, the ideal reporter being a small monomeric enzyme.

The ability to generalize the PCA strategy was taught in the '964 patent and in the Michnick publications. The principle of PCA was designed such that reconstitution of enzyme activity from fragment pairs would be interaction-dependent. As explained in Michnick reference (1), p. 12146, third paragraph: "There is an interesting symmetry to the system described here: the fact that the oligomerization domains are absolutely required for reconstitution means that the reconstitution of activity itself is a detector of the oligomerization domain interactions".

Summary of WO 00/71702 vs. Prior Art

In this section we analyze the three key elements described in the Summary of WO 01/71702, in comparison with the prior art.

1. "Compositions and methods are provided for identifying interactions between polypeptides using an interaction-dependent protein association system. The system is characterized by using fragment pairs comprised of a first and a second member that functionally reassemble into a marker protein having a directly detectable signal, such as a visible phenotypic change or antibiotic resistance." Comparison with US 6,270,964 shows that this description is identical to PCA. See for example the first sentence of the Abstract of the '964 patent: "We describe a strategy for designing and implementing protein-fragment complementation assays to detect biomolecular interactions in vivo and in vitro". In Section 3 line 40 of the '964 patent: "In a preferred embodiment, the instant invention seeks to provide an oligomerization-assisted

complementation of fragments of monomeric enzymes that require no other proteins for the detection of their activity". In Section 4 line 49 of the '964 patent: "Additional assays, including those based on enzymes that can act as dominant or recessive drug selection or metabolic salvage pathways are disclosed". Many examples of suitable marker proteins are given, including examples of marker proteins conferring a visible phenotypic change or antibiotic resistance. For example in Section 14 line 43 of the '964 patent: "The overall strategy is described for the case of aminoglycoside kinase, an example of an antibiotic resistance marker that can be used for dominant selection of prokaryotic cells such as E. coli or eukaryotic cells such as yeast or mammalian cell lines." Also clear in the '964 patent is that PCA is not limited to any specific enzyme. In Section 16 line 3 of the '964 patent: "This strategy can be used to develop any PCA based on enzymes that impart dominant or recessive selection to a drug or toxin or to enzymes that produce a colored or fluorescent product".

2. "The fragment complementation system of the present invention involves co-expression in a host cell of a first and a second oligopeptide, where each is a fusion protein separated by a flexible polypeptide linker with a member of the marker protein fragment pair. Binding of the first oligopeptide to the second oligopeptide results in the functional reconstitution of the fragment pair into a marker protein, and the interacting first and second oligopeptides are identified by isolating and sequencing plasmids from a host cell that displays a directly detectable signal indicative of the marker protein."

Comparison with US 6,270,964 shows that this description is identical to PCA. See for example the Summary, section 4 line 31 of the '964 patent: "Using molecular biology

techniques, the chosen fragments are subcloned, and to the 5' ends of each, proteins that either are known or thought to interact are fused. Co-transfection or transformation of these DNA constructs into cells is carried out. Reassembly of the probe protein or enzyme from its fragments is catalyzed by the binding of the test proteins to each other, and reconstitution is observed with some assay. That is, observation of reconstituted enzyme activity must be a measure of the interaction of the fused proteins". In Section 3 line 48 of the '964 patent: "This assay requires no additional endogenous factors for detecting specific protein-protein interactions and can be conveniently extended to screening cDNA, nucleic acid, small molecule or protein design libraries for molecular interactions".

3. "The invention also provides for efficient methods of finding functional fragment pairs of a marker protein that involve identifying functional breakpoints within flexible loops using tertiary or secondary structural information."

The use of tertiary or secondary structural information to identify functional breakpoints is an element of rational design to create a PCA, and strategies for the use of rational design of fragments were described extensively in the '964 patent. For example in Section 16 line 14 of the '964 patent: "Knowledge of the enzyme structure can be used to render a more efficient way of using molecular evolution to design a PCA". As further described in the '964 patent, rational design was used to design a PCA based on DHFR and the general principles of fragment design were described, section 10 line 42: "We chose to cleave mDHFR between fragments [1,2] and [3], at residue 107, so as to cause minimal disruption of the active site and NADPH cofactor binding sites. The native N-terminus of mDHFR and the novel N-terminus created by cleavage

occur on the same surface of the enzyme". In addition: "...if the structure of an enzyme were known if would be possible to design fragments of the enzyme to ensure that the reassembled fragments would be active and to introduce mutations to alter the stringency of detection of reassembly."

WO 00/71702: Analysis of Claims

Here we analyze selected claims of PCT WO 00/71702 in relation to the prior art.

00/71702 Claim 1: Method of Identifying a Functional Fragment Pair: "A method of identifying a functional fragment pair in a protein, said method comprising:
preparing fragments of a marker protein wherein each fragment has a break-point terminus within a solvent exposed loop of said marker protein, wherein the N or C terminal residue of each C or N terminal fragment, respectively, constitutes said break-point terminus, to obtain a marker fragment library; expressing in a multiplicity of host cells, members of said marker fragment library; isolating host cells expressing said marker protein as indicative of a cell containing a first member and a second member of a fragment pair which have formed a functionally reconstituted said marker protein, whereby said functional fragment pair is identified."

This claim is directed toward preparing a library of fragments in order to identify a functional fragment pair in a protein, that is, a fragment pair capable of reconstituting the activity of the marker protein. Such methods of identifying functional fragment pairs were described in the '964 patent, for example Section 14-16 describes such a strategy for a PCA using

aminoglycoside kinase : "Generation of a library of AK fragments based on products of exonuclease digestion"; "Screening for AK activity"; and "Directed evolution of optimal AK fragments using DNA shuffling". The identification of a functional fragment pair is described beginning in Section 15 line 62 of the '964 patent: "Those clones showing the maximal resistance to G418 are then selected and if maximum resistance or greater is reached the evolution is terminated... Finally, optimal fragments are sequenced and physical properties and enzymatic activity are assessed... This strategy can be used to develop any PCA based on enzymes that impart dominant or recessive selection to a drug or toxin or to enzymes that produce a colored or fluorescent product".

Claim 1 of 00/71702 requires the use of PCA to create and identify functional fragment pairs of marker proteins. For example Claim 2 of the '964 patent covers: "A method for detecting biomolecular interactions said method comprising: (a) selecting an appropriate enzyme reporter molecule; (b) effecting fragmentation of said enzyme reporter molecule such that said fragmentation results in reversible loss of reporter function; (c) fusing or attaching fragments of said enzyme reporter molecule separately to other molecules; (d) reassociating said enzyme reporter fragments through interactions of the molecules that are fused or attached to said fragments; and (e) detecting the activity of said enzyme reporter molecule." Claim 8 of the '964 patent covers: "The method of claim 2 wherein said fragmentation is effected by a method selected from the group consisting of genetic manipulation, synthetic chemistry or de novo synthesis, photochemical or enzymatic cleavage, and proteolytic or hydrolytic chemistry". Claim 25 of the '964 patent covers: "A method of detecting kinetics of protein assembly

comprising the method of any one of claims 1, 2, 9, 12 or 18 wherein time dependent reconstitution of enzyme activity is the detecting means".

00/71702 Claim 9: Method of Identifying Interacting Oligopeptides from a Library:

"A method of identifying a second oligopeptide to which a first oligopeptide binds, said method comprising: co-expressing in a multiplicity of host cells said first oligopeptide and said second oligopeptide wherein said second oligopeptide is encoded by a member of a library, each as a fusion protein with a first member and a second member of a fragment pair of a marker protein, respectively, obtained according to the method of Claim 1, wherein binding of said first oligopeptide to said second oligopeptide results in the functional reassembly of said marker protein; isolating host cells expressing said marker protein as indicative of a cell containing a first oligopeptide and a second oligopeptide which have interacted; and sequencing plasmids containing expression cassettes coding for said fusion proteins whereby said second oligopeptide to which said first oligopeptide binds is identified." This claim describes the use of PCA for library screening, which is not new; methods of library screening using PCA are specifically taught and claimed in US 6,270,964. Claim 2 of the '964 patent covers: "A method for detecting biomolecular interactions said method comprising: (a) selecting an appropriate enzyme reporter molecule; (b) effecting fragmentation of said enzyme reporter molecule such that said fragmentation results in reversible loss of reporter function; (c) fusing or attaching fragments of said enzyme reporter molecule separately to other molecules; (d) reassociating said enzyme reporter fragments through interactions of the molecules that are fused or attached to said fragments; and (e) detecting the activity of said enzyme reporter molecule." Claim 26 of the '964 patent covers: "A method of screening a cDNA library comprising performing an enzyme

complementation assay wherein said assay detection means is reconstitution of enzyme activity". Claim 35 of the '964 patent covers: "A method of screening a cDNA library comprising the method of any one of claims 1, 2, 9, 12 or 18".

The use of PCA to screen either oligopeptide libraries or cDNA libraries is described in US 6,270,964. The application to screening of an oligopeptide library is depicted in Figure 7 of US 6,270,964 and described as follows: "Figure 7 illustrates the results of a protein engineering application of the mDHFR bacterial PCA. Two semi-random leucine zipper libraries were created and each inserted N-terminal to one of the mDHFR fragments. Co-transformation of the resulting zipper-DHFR fragment libraries in E. Coli and p;lating on selective medium allowed for survival of clones harboring successfully interacting leucine zippers". This application was also documented in References 5 and 7 by Michnick and coworkers . A further example of library screening was given in Example 2 (b) of US 6,270,964: "Modification of the bacterial survival assay for library screening", and depicted in Figure 5; these are exactly the steps followed in WO 00/71702. Two publications (References 5 and 7) describe library-vs.-library screening in bacteria using PCA.

00/71702 Claims 10, 11 and 26: Signal Peptides: "The method according to Claim 9, wherein each of said fusion proteins further comprises a signal peptide." "The method according to Claim 10, wherein said signal peptide provides for translocation to the periplasm of a bacterial cell". "The fragment complementation system according to Claim 25, wherein said first and said second oligopeptide further comprise a signal peptide". The use of signal peptides in conjunction with PCA was taught in US 6,270,964. Specifically in Section 5 line 55 of the '964

patent: "Constructs could be designed for targeting the PCA fusions to specific compartments in the cell by addition of signaling peptide sequences ."

00/71702 Claim 22: Fragment Complementation System: "A fragment complementation system, said system comprising: a first oligopeptide comprising an N-terminal fragment with a C-terminal breakpoint, and a second oligopeptide comprising a C-terminal fragment with a N-terminal breakpoint, wherein said N-terminal fragment and said C-terminal fragment each are derived from a marker protein and reassemble to form a functionally reconstituted marker protein." This claim reads directly on US 6,270, 964 Claim 1 :: "Protein fragment complementation assays for the detection of molecular interactions comprising a reassembly of separate fragments of an enzyme, wherein reassembly of said fragments is operated by the interaction of molecular domains fused to each enzyme fragment, and wherein reassembly of the enzyme fragments is independent of other molecular processes and wherein said reassembly is detected by means of reconstitution of activity of said enzyme". Claim 2 of the '964 patent also covers: "A method for detecting biomolecular interactions said method comprising: (a) selecting an appropriate enzyme reporter molecule; (b) effecting fragmentation of said enzyme reporter molecule such that said fragmentation results in reversible loss of reporter function; (c) fusing or attaching fragments of said enzyme reporter molecule separately to other molecules; (d) reassociating said enzyme reporter fragments through interactions of the molecules that are fused or attached to said fragments; and (e) detecting the activity of said enzyme reporter molecule."

00/71702 Claim 25: Fragment Complementation System: "A fragment complementation system, said system comprising: a first oligopeptide comprising an N-terminal fragment fused through a break-point to a flexible polypeptide linker and a first interactor domain; and a second oligopeptide comprising a second interactor domain and a flexible polypeptide linker fused through a break-point to a C-terminal fragment, wherein said N-terminal fragment and said C-terminal fragment are obtained according to the method of Claim 1, and wherein said N-terminal and said C-terminal fragment functionally reconstitute said marker protein only upon binding of said first interactor domain with said second interactor domain." This claim also reads directly on Claim 1 of the '964 patent, which covers: "Protein fragment complementation assays for the detection of molecular interactions comprising a reassembly of separate fragments of an enzyme, wherein reassembly of said fragments is operated by the interaction of molecular domains fused to each enzyme fragment, and wherein reassembly of the enzyme fragments is independent of other molecular processes and wherein said reassembly is detected by means of reconstitution of activity of said enzyme".

00/71702 Claim 35: Fragment Complementation System: "A fragment complementation system, said system comprising: a first oligopeptide comprising an N-terminal fragment of a β-lactamase fused through a breakpoint to a flexible polypeptide linker and a first interactor domain; and a second oligopeptide comprising a second interactor domain and a flexible polypeptide linker fused through a breakpoint to a C-terminal fragment of a β-lactamase, wherein said N-terminal and said C-terminal fragment functionally reconstitute said β-lactamase upon binding of said first interactor domain with said second interactor domain." This Claim reads directly on Claims 1, 12, 15, 28, 2 and 6 of the '964 patent. Claim 1 of the '964 patent

covers: "Protein fragment complementation assays for the detection of molecular interactions comprising a reassembly of separate fragments of an enzyme, wherein reassembly of said fragments is operated by the interaction of molecular domains fused to each enzyme fragment, and wherein reassembly of the enzyme fragments is independent of other molecular processes and wherein said reassembly is detected by means of reconstitution of activity of said enzyme." Claim 12 of the '964 patent covers: "A method comprising an assay where fragments of a first enzyme molecule are fused to a second molecule and fragment association is detected by reconstitution of the first enzyme molecule's activity." Claim 15 of the '964 patent covers: "A composition comprising complementary fragments of a first enzyme molecule that exhibits a detectable activity when associated, wherein each fragment is fused to a separate molecule." Claim 28 of the '964 patent covers: A method for detecting protein-protein interactions in living organisms and/or cells, which method comprises: (a) synthesizing probe protein fragments from an enzyme which enables dominant selection by dissecting the gene coding for the enzyme into at least two fragments; (b) constructing fusion proteins consisting of the probe protein fragments linked to protein domains that are to be tested for interactions; (c) coexpressing the fusion proteins; and (d) detecting reconstitution of enzyme activity. Claim 2 of the '964 patent covers: "A method for detecting biomolecular interactions said method comprising: (a) selecting an appropriate enzyme reporter molecule; (b) effecting fragmentation of said enzyme reporter molecule such that said fragmentation results in reversible loss of reporter function; (c) fusing or attaching fragments of said enzyme reporter molecule separately to other molecules; (d) reassociating said enzyme reporter fragments through interactions of the molecules that are fused or attached to said fragments; and (e) detecting the activity of said enzyme reporter molecule."

Claim 6 of the '964 patent covers: "The method of claim 2 wherein said reporter molecule is a catalytic molecule."

In sum, WO 00/71702 describes PCA. The ability to apply PCA to a wide variety of reporters, cell types, cell compartments, and biological applications, is taught in the '964 patent. This includes examples of the choice, characteristics, and methods for the engineering of PCA reporters. In section 9 line 59 of the '964 patent: "In designing a protein-fragment complementation assay (PCA) we sought to identify an enzyme for which the following is true: 1) "An enzyme that is relatively small and monomeric" (this is true for β-lactamase), 2) "for which structural and functional information exists" (this is true for β-lactamase), 3) "for which simple assays exist for both in vivo and in vitro measurements" (this is true for β-lactamase), and 4) "for which overexpression in eukaryotic and prokaryotic cells has been demonstrated" (this is true for β-lactamase). In section 8 line 18 of the '964 patent: "It should be understood that the instant invention is not limited to the PCAs presented here, as numerous other enzymes can be selected and used in accordance with the teachings of the present invention." In section 7 line 49 of the '964 patent: "In a further embodiment, the invention provides a method for detecting protein-protein interactions in living organisms and or cells, which method comprises: (a) synthesizing probe protein fragments from an enzyme which enables dominant selection by dissecting the gene coding for the enzyme into at least two fragments; (b) constructing fusion proteins with one or more molecules that are to be tested for interactions; (c) fusing the proteins obtained in (b) with one or more of the probe fragments; (d) co-expressing the fusion proteins; and (e) detecting the reconstitution of enzyme activity." In sum, the use of β-lactamase for PCA

fits the criteria exactly as taught in the '964 patent including the use of a marker protein enabling dominant selection to provide a detectable phenotype.

00/71702 Claim 42: Compositions: "An expression cassette comprising: as operably linked components in the direction of transcription nucleotide sequences encoding for: (i) a promoter functional in a host cell; (ii) a polypeptide interactor domain; (iii) a flexible polypeptide linker; and (iv) a C-terminal fragment of a marker protein that provides for a selectable phenotype." **00/71702 Claim 43:** "An expression cassette comprising: as operably linked components in the direction of transcription nucleotide sequences encoding for: (i) a promoter functional in a host cell; (ii) a polypeptide interactor domain; (iii) a flexible polypeptide linker; and (iv) an N-terminal fragment of a marker protein that provides for a selectable phenotype." These claims describe the nucleic acid constructs exactly as taught and claimed in the '964 patent. Claims 42 and 43 read directly on Claims 17 and 21 of the '964 patent. Claim 17 covers: "A composition comprising a nucleic acid molecule coding for an enzyme fusion product, which molecule comprises sequences coding for a product selected from the group consisting of: (a) a first enzyme fusion product comprising: 1) fragments of a first enzyme molecule whose fragments can exhibit a detectable activity when associated and 2) a second molecule fused to the fragment of the first molecule; (b) a second fusion product comprising 1) a second fragment of said first enzyme molecule and 2) a second or third molecule; and (c) both (a) and (b)." Claim 21 of the '964 patent covers: "A composition comprising a nucleic acid molecule coding for an enzyme fusion product, which molecule comprises sequences coding for either: (a) a first fusion product comprising: 1) a first fragment of an enzyme molecule whose fragments exhibit a detectable activity when associated and 2) a

first protein-protein interacting domain; or (b) a second fusion product comprising 1) a second fragment of said enzyme molecule and 2) a second protein-protein interacting domain; or (c) both (a) and (b)." It should be noted that the use of a flexible polypeptide linker between the interactor domain and the fragment of the marker protein is not new; it was taught by Michnick et al. who used flexible polypeptide linkers with the DHFR PCA. Such constructs are described in the '964 patent (see '964, figure 2) and in the published articles (see reference 2 p. 12142 "Construction of a flexible linker and subcloning of F[1,2] and F[3]"; such constructs are also shown in figure 1 of reference 2; and in Methods in Enzymology which further describes PCA's for DHFR, GAR transformylase, aminoglycoside phosphotransferase and green fluorescent protein).

Summary of WO 01/51629 vs. Prior Art

Two components of the proposed Balint invention are described:

The first component, shown in Figure 1B, is identical to that of WO 00/71702 and shows PCA, in which fragment pairs of a marker protein are used to generate an interaction-dependent signal. The "Brief Description of the Specific Embodiments" for this first component is identical to the "Brief Description of the Specific Embodiments" given in WO 00/71702 which, as discussed above, describes PCA. Moreover, Examples 1- 9 of the WO 01/51629 are identical to Examples 1-9 of WO 00/71702 which describe a PCA using β-lactamase. Example 12 of WO 01/51629 is identical to Example 10 of WO 00/71702 which describes a PCA using neomycin phosphotransferase. Example 10 of WO 01/51629 describes PCA as applied to antibody-directed enzyme prodrug therapy.

In the second component, shown in Figure 1A of WO 01/51629, no fragment pairs are created - instead, a circularly permuted marker protein is inserted in frame between two interacting oligopeptides in a single expression cassette. Although the title of the patent application is "Circularly permutated interaction-dependent proteins", there is only one example that describes that claimed invention, namely Example 11: "Ligand-dependent activation of Circular Permutations of β-Lactamase".

The language of the claims of WO 01/51629 is such that it is not obvious whether the applicants intend to claim both PCA and circularly permutated interaction-dependent proteins, or only the latter. For example claim 1 of WO 01/51629 refers to "functional reassembly" of a marker protein. The word "reassembly" suggests that discrete elements of the marker protein come together, which is only the case for PCA in which a fragment pair functionally reassembles to generate an active reporter.

However, the description given in WO 01/51629 uses the term "reassembly" to refer both to PCA and to circularly-permutated proteins: "The methods detect the interaction of a first known or unknown interactor domain with a second unknown interactor domain, or a first and second interactor domain and a third known or unknown interactor domain, **by bringing into close proximity members of a fragment pair of a marker protein or a circular permutation of a marker protein, such that the parent marker protein is reassembled** to its original functionality, and such that **reassembly** requires the prior interaction of the heterologous interactor domains. A first interaction-dependent enzyme activation system described herein is

characterized by N-terminal and C-terminal **fragment members** that comprise fragment pairs which are derived from, and can **functionally reassemble** into a marker protein that provides for a directly detectable signal that does not involve downstream steps necessary for recognition". This description, which incorporates both components of the claimed invention and utilizes the words "functional reassembly" that are later repeated in the claims, is identical to the description of PCA in the specifications and claims of US 6,270,964 (see claims language on next page). In contrast, the concept of reassembly has no meaning in the case of "circularly permutated interaction-dependent proteins", in which a whole β-lactamase protein is inserted between other proteins or domains. Moreover, although the applicants have combined the two components in describing their claimed invention, in their specifications (p. 67 line 22) they go on to explain that there are two different mechanisms for the two different components of their claimed invention: "The proposed mechanism by which such CPs (circular permutations) are activated is roughly the opposite of that by which interaction-dependent fragment complementation is believed to occur. In the latter, the heterologous interaction docks the fragments long enough to allow them to refold into the active conformation. However, in the case of the CP, it is transient folding of the CP which allows the interactors to make contact, and the latter then traps the CP in an active conformation" .

The fragment complementation systems of WO 01/51629 read on Claims 1, 2, 6, 8, 9, 10, 11,13, 14,15,16,17,18, 19, 20, 21, 22, 28, 32, 33, and 38 of US 6,270,964. Several of the '964 patent claims are reproduced here.

Claim 1 of 6,270,964: "Protein fragment complementation assays for the detection of molecular interactions comprising a reassembly of separate fragments of an enzyme, wherein reassembly of said fragments is operated by the interaction of molecular domains fused to each enzyme fragment, and wherein reassembly of the enzyme fragments is independent of other molecular processes and wherein said reassembly is detected by means of reconstitution of activity of said enzyme".

Claim 2 of US 6,270,964: "A method for detecting biomolecular interactions said method comprising: (a) selecting an appropriate enzyme reporter molecule; (b) effecting fragmentation of said enzyme reporter molecule such that said fragmentation results in reversible loss of reporter function; (c) fusing or attaching fragments of said enzyme reporter molecule separately to other molecules; (d) reassociating said enzyme reporter fragments through interactions of the molecules that are fused or attached to said fragments; and (e) detecting the activity of said enzyme reporter molecule."

Claim 6 of US 6,270,964: The method of claim 2 wherein said reporter molecule is a catalytic molecule".

Ligand-Dependent Activation of Circular Permutations of β-Lactamase (Example 11 of WO 01/51629)

As mentioned above, this is the only example given in either WO 00/71702 or WO 01/51629 that does NOT utilize PCA. It is unclear whether example 11 provides enablement, or describes anything new. The examiner is directed to several references on circular permutations of proteins, including examples in which a marker protein is inserted between domains of

interacting proteins and changes in the activity of β-lactamase are used to detect changes in the associated proteins. Most notable in this regard is the 1999 work by Betton et al. in reference 12 entitled "Creating a bifunctional protein by insertion of β-lactamase into the maltodextrin-binding protein". Betton et al. created fully active bifunctional proteins by implanting the monomeric enzyme β-lactamase into permissive sites in the maltodextrin-binding protein and were able to show enzyme stabilization via specific ligands in a manner similar to that described in example 11 of WO 01/51629. Note in particular Figure 1 of reference 12, which shows a construction employing β-lactamase that is identical to that shown in Figure 1A of WO 01/51629.

Summary

WO 00/71702 and WO 01/51629 are virtually identical in specifications and examples, except for one example - example 11 of WO 01/51629 - which describes a circularly-permutated β-lactamase. Analysis of the specifications and examples of both WO 00/71702 and WO 01/51629 shows that the applicants are using protein-fragment complementation assays (PCA) including methods, compositions, and applications identical to those taught in US 6,270,964 (Michnick et al.) and in the references provided below.

Respectfully submitted,

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CERTIFICATE OF SERVICE

I hereby certify that a copy of the foregoing PROTEST UNDER RULE 37 C.F.R. § 1.291 was served on Applicant's representative by UPS on March 22, 2002, addressed as follows:

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Date: 3/22/02

Stella Peña